GROWTH OF EPHEMERAL PLANTS FOR BIOREGULANT RESEARCH

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Recently there is increased interest in growth regulators and herbicides to modify plant growth and control weeds. Also, a vast number of chemicals are available for testing of biological activity. There is a necessity for broad primary screening methods that give maximum information in a short time concerning potential plant regulating chemicals.

Techniques for rapid screening have been developed using tissue culture, algae, and higher plant species. Detection of some types of bioactivity is possible by in vitro methods. However, completely controlled and unnatural growing conditions do not necessarily reflect the response of plants under outdoor field conditions. The reaction of plants that have different environmental requirements for growth and reproduction cannot be examined satisfactorily with a single species treated with chemicals and grown either under tissue culture or near tissue culture conditions.

In consideration of the limitations of available methods for detecting plant bioregulants, considerable thought and research was devoted to developing a rapid, space-conserving method for plant regulating chemicals. The original objectives were to domesticate plants that could complete their life cycle in 30 days or less, and that could be cultured in large numbers in confined space.

In initiating this project, it was recognized that the type of plants needed were short-lived, relatively small annuals (the type commonly referred to as ephemerals). Also, it was deemed necessary to grow the plants under standardized conditions in controlled environmental facilities. A search for potential test plants was made in four environments in southern California. These areas included the Mojave Desert, the San Gabriel and San Bernardino Mountain ranges, the Coastal Sage and grassland, and various cultivated areas in the region. The initial search was conducted during the flowering period of annuals that grew in each particular environment. Records were maintained concerning natural environmental and edaphic conditions under which the plants and set seed grew. All annuals that were small at maturity were marked and observed weekly. Seeds were collected during the normal period of dissemination, and type specimens were collected and mounted for future reference. Seeds from nearly 200 species were collected from the four natural environmental areas for research under controlled environmental conditions.

Germination tests were conducted on all seeds within 90 days after collection. Lots of 100 seeds were placed in petri dishes

between layers of filter paper and kept damp with distilled water. Initial tests were made in the dark at various constant temperatures ranging from 3 C to 27 C. The number of seeds germinating within a 20-day period was recorded, and this was considered to be the percent germination under the specific conditions used. Germination of 70% or better under any of the temperatures used was considered satisfactory. Seeds that did not have a high germination rate were subjected to specific pretreatments as follows: 50 C dry heat for seven days, 1 C under moist conditions in the dark for 20 days, a combination of the two treatments with the heat treatment preceding the cold treatment, running water for 48 hours, and scarification. Species whose seeds did not germinate more than 40% under any of the above treatments were discarded.

A series of experiments was initiated to develop and establish domestication conditions and practices for candidate bioregulant test species. The common garden weed, Senecio vulgare L., was used for initial evaluation of the system. This species was chosen because of the relative ease of obtaining seeds, culturing plants, and growing them to maturity. In the initial experiments involving temperature and photoperiod, the plants were grown in 150 ml styrofoam cups with holes punched in the bottom for drainage. The growing medium was washed river sand. The temperature regimes were 23 C day and 17 C night, 23 C day and 13 C night, 29 C day and 17 C night, and 29 C day and 23 C night. All temperature patterns were used with a 12-hr photoperiod and a 16-hr photoperiod. The S. vulgare strain used grew equally well with a single daily watering under either 23 C day and 17 C night, or 29 C day and 17 C night with either photoperiod. Therefore further experimentation with S. vulgare was confined to these specific culture temperatures and photoperiods.

Nutrients for ephemeral plants were examined from the standpoint of satisfactory plant growth, ease of application, and ready availability of the nutrient mixture in adequate quantities for large scale research projects. The complete inorganic fertilizer with a trade name of Kapco (15-30-15) was chosen as a standard. Two concentrations of Kapco, 10 g/l and 0.5 g/l were used. Other nutrient mixtures are available which are equally useful. The fertilizer solutions were applied in the place of water once a week, three times a week, and daily. Six cultures with three plants were used in each test replication. Controls were always used. Over 70% of the Senecio plants bloomed in 20 days or less with the nutrient solution, sand, and standard environmental conditions. All plants had excellent vegetative growth when 0.5 g/l of nutrient was applied daily with no other water applied. All other applications of Kapco resulted in less satisfactory plant growth. When 10 g/l was applied daily, all plants died. As a result of the data from the nutrient research, a practice of watering plants daily with 0.5 g/l Kapco solution was adopted.

Potting media were examined and sand was selected as the most desirable type. Therefore, washed river sand and graded silica sand were compared. Washed river sand varies considerably as to particle size, waterholding capacity, type of rock mixture, organic matter, and nutrients. Silica sand is a prepared mixture that is graded, with a consistent mineral content. An experiment was established with blocks of 30 plants each and 3 plants per culture; some blocks were planted in river sand, and the others in 1.6mm dia. silica sand. Plants were grown at 23 C day, 17 C night temperatures with a 12-hr photoperiod. All blocks of plants received daily Kapco nutrient treatments at 0.5 g/l. Twenty-six days after planting, all plants growing in river sand had flower buds, while only four plants in silica sand had flower buds. Vegetatively, plants in river sand were larger and more robust in appearance than those plants in silica sand.

Data from previous experiments demonstrated the presence of substantial variability of ephemeral plant growth at the various temperatures, photoperiods, culture media, and nutrient levels. Because of the possible variability of plant growth, standardized method of plant culture was established. Three groups of 5-10 seeds each were planted in washed river sand in 150 ml styrofoam cups with holes punched at the base for drainage. When the plants were in the cotyledon stage, they were thinned to 1 to 3 (depending on species), leaving the single strongest uniform plants. Plants were watered daily with Kapco, 0.5 g/l. Initial temperatures of 23 C day, 17 C night with a 12-hr photoperiod and temperatures of 29 C day, 17 C night with a 16-hr photoperiod were used. Plants were evaluated for rigor, growth pattern, size, time to flower, bud appearance, time to anthesis, time to seed maturity, and uniformity of all observed factors.

From the research outlined above, the following species selected as best suited for further investigation research; the group contains a wide range of natural morphological and physiological variations.

Chenopodium humile Hook is one of the common goosefoot group that are major weeds in temperature regions. C. humile grows at high elevations in moist alkaline places and is characterized by a decumbent rather than erect growth pattern. It is a short-day plant, blooming on a 12-hr photoperiod and remaining vegetative on a 16-hr photoperiod. When grown on a 12-hr photoperiod, plants bloom in as few as 14 days after planting the seed, when they are about 3cm in height. C. humile has a specific value in that it may be grown on a 16-hr photoperiod and treated by foliar chemical applications when desired plant size is reached. Then, the block may be divided, retaining one-half on the 16-hr photoperiod and the other one-half moved to a 12-hr photoperiod where flowering is induced. In this manner, in addition to observing the effects of chemicals on vegetative characters, observations may be made on

their effect of floral primordia initiation, development, and seed set. This series of vegetative and floral observations can be made in less than 30 days.

Lepidium flavum Torr. is one of the peppergrass group in the mustard family. L. flavum is native to the southwestern deserts of California. It is a winter annual and grows well at low temperatures. Therefore, it is not a satisfactory experimental ephemeral plant at higher temperatures. The plant is a day neutral rosette type with a central flower staff developing about 15 days after planting. It is a candidate experimental plant for screening of dwarfing and retarding plant growth regulators. L. flavum blooms in less than 30 days under the standard experimental conditions used in this research. Different plants of this species do not develop as uniformly as do the other species studied; it is, however, the best plant of a true rosette type investigated in the project.

Poa annua L. (Annual bluegrass) is practically a worldwide weed. It is an excellent grass for testing purposes, it germinates and grows rapidly. However, these are distinct P. annua strains. Seeds collected around Riverside, California, are from two distinct strains. One strain is heavier, robust, and flowers in 30-40 days. The other is slighter, with few stalks, and flowers under the conditions specified above in less than 20 days. The later strain has been isolated and can be maintained by growing seed plants in the greenhouse. Annual bluegrass is a typical grass and has the terminal meristem well protected from physical contact with materials applied during foliar treatment. It is good for screening for growth regulators and herbicides specific to grasses.

Rorippa obtusa (Nutt.) Britton belongs to the yellow cress group and a close relative of water cress. R. obtussa grows in damp places at higher elevations, particularly on lake shores and along streams. Although not common, it is generally distributed across the United States. It is a small, rapid-growing partial rosette type plant that flowers under the standard conditions of this research, in less than 25 days after planting. The plants are very consistent in growth habits and have excellent plant form; as such, they are of particular value in observing and separating multiple effects of plant regulating chemicals.

Rumex fueginus Phi. (Golden Dock) is an annual species of the Sorrel or Dock Family. It grows in wet and often brackish places. Although it is widespread across North America, it is only common on the Pacific Coast. R. fueginus is probably the smallest and most rapidly-growing species of the genus. It is a long-day plant, flowering on a 16-hr photoperiod and remaining vegetative on a 12-hr photoperiod. This photoperiod response is associated with day length and flower stalk initiation. If grown on a 16-hr photoperiod, R. fueginus can be induced to flower from seeding in about

30 days. It is a dicotyledonous plant that has a terminal meristem protected by an envelope of leaves and, hence, like the grasses, is protected from direct contact by foliar applied chemicals. Further, it is an acaulescent-type plant with the central stalk being initiated only for flowering.

Schismus arabicus Nees. is a low-tufted annual grass resembling the larger bunch grasses. It is native of Southest Asia and is widespread in the Mojave Desert. Under uniform growing conditions of this research, it matures rapidly but does not produce flowers until it is grown for more than three months. S. arabicus is a good experimental grass to observe possible dwarfing and lethal chemicals applied to grasses in a vegetative condition. Satisfactory seed germination occurs only after seeds are treated with running water. If exposed to running water for 24-hrs, germination of 90% or better will occur in one day. The characteristics of S. arabicus allow for exceptional uniformity of testing material.

Senecio vulgaris L. (Common Groundsel) is a common weed in gardens and waste places; it is practically worldwide in distribution. It is a well-rounded, multiple branched plant of excellent form when grown under a 12-hr photoperiod and a cool temperature. At higher temperatures and a 16-hr photoperiod, it generally forms a central stalk and does not produce the same multi-branched, well-rounded condition that exists at lower temperatures. Its terminal meristem and any axillary meristems that may be in active growth are unprotected and are directly contacted by any material that is generally sprayed on the plant. In this respect, it is a valuable partner of R. fueginus in comparing effects of treatment associated with transport of the experimental material applied to the foliage. S. vulgaris blooms in about 20 days from seeding at the longer photoperiod and higher temperatures and in about 30 days at the shorter photoperiod and lower temperature.

To determine the usefulness of groups of ephemeral plants in research, plants of C. humile, P. annua, R. fueginus, and S. vulgaris were treated with a series of growth and herbicides, at 1000 ppm, 500 ppm, 100 ppm, and 50 ppm. Two ml of test solution was sprayed on a container of three plants. Application was made 48 hours prior to the time that flower buds would be visible under the growing conditions used. A series of such compounds, including NAA, 2,4-D, phosphon, kinetin, MH, alar, ethrel, cycocel, and atrazine were applied. In most cases, the ephemeral plants reacted to the foliar applied chemicals in the same way that larger, longer-lived plants do.

Other research to determine response of ephemeral plants after treatment of the growing medium and uptake of the chemicals through the root system was conducted. The technique is useful since it simulates soil treatment and the concentration and time of application to the medium can be controlled. Several growth regulators

and herbicides listed above were applied to the growing medium with the watering solution. Typical responses resulted that have been noted for other plants in previous research widely reported in the literature.

An example of the use of ephemeral plants in bioregulant research follows: C. humile plants were placed in a controlled environment chamber with the long-day conditions (16-hr pp) and R. fueginus in the growth chamber with the short-day conditions (12-hr pp). At 15 to 18 days from seeding, the plants were in flower bud stage. Some were treated with growth regulators listed above and shifted to the other photoperiod, while others were left with the same photoperiod. After shift in photoperiod, other previously untreated plants were treated by foliar applicaiton of the same compounds. In 25 to 30 days from planting, the controls of those plants under the floral primordia initiating photoperiod produced large visible flowers. Vegetative effects of the test compounds were readily observable. Chemicals that affected either C. humile or R. fueginus plants were then used to treat P. annua, R. obtusa, and S. vulgare. P. annua and S. vulgare grown at 23 C day, 17 C night temperatures with a 12-hr pp, and R. obtusa grown at 29 C day, 17 C night temperatures with a 16-hr pp. were treated 48 hours prior to the average time for flower bud appearance and at chemical concentrations of 1000 ppm, 500 ppm, 100 ppm, and 50 ppm.

An adequate supply of seed of the species discussed as test plants here is easily maintained in a small greenhouse area. All of the plants have been grown in 150 ml styrofoam cups with river sand and nutrient solution, and in larger containers with the usual potting mixtures under average greenhouse conditions. No other conditions are required except in the case of C. humile and R. fueginus. These two species should be grown in a greenhouse or growth chamber under a vegetative photoperiod until an adequate-sized plant is obtained. At this point, they should be transferred to a floral primordia initiating photoperiod. Seeds are collected from greenhouse grown plants and stored for subsequent research.

If treatments are carefully timed, the method of research outlined here provides maximum information concerning vegetative effects, floral primordia initiation effects, and seed production effects in 30 days or less. The method produces information on the effect of a given compound on all phases of the morphological development of angiosperm over a wide taxonomial range of plants. The method is economical since many cultures of plants can be grown in limited space.